



Susceptibility of Tree Seedlings to Biotic and Abiotic Hazards in the Understory of a Moist Tropical Forest in Panama

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ABSTRACT

We evaluated temporal patterns of seedling survival of eight Neotropical tree species generated under multiple abiotic and biotic hazards (vertebrates, disease, litterfall) in the forest understory on Barro Colorado Island, Panama. Seedlings were transplanted at first leaf expansion in low densities along a 6-km transect and damage and mortality were recorded for 1 yr. We also planted and monitored small and large artificial seedlings to estimate physical disturbance regimes. During 0–2 mo after transplant, vertebrate consumers of reserve cotyledons caused high mortality of real seedlings, but little damage to artificial seedlings. On real seedlings after 2 mo, disease became an important agent of mortality, despite a decrease in overall mortality rates. Damage by litterfall remained relatively low during the 1-yr study period. Survival ranks among species showed ontogenetic shifts over time, as species changed susceptibility to the mortality agents. Survival after 2 mo was positively correlated with stem toughness, not because species with tough stems were less likely to receive mechanical damage, but because they survived better after receiving mechanical damage. Within each transplant station, artificial seedlings were not good predictors of litterfall damage experienced by real seedlings. Forest-wide litterfall damage level, however, was similar for both real and artificial seedlings (*ca* 10%/yr), a moderate level compared to other tropical forests. In conclusion, species traits including biomechanical traits interact to create complex temporal patterns of first year seedling survival, resulting in ontogenetic shifts that largely reflect changes in the relative importance of vertebrate consumers relative to other hazards.

Key words: artificial seedlings; Barro Colorado Island; herbivory; litterfall; ontogenetic shifts; stem biomechanics; tropical tree seedling survival; vertebrate activity.

VARIOUS MORTALITY AGENTS INFLUENCE SPATIAL DISTRIBUTIONS OF SEEDLINGS in relation to environmental heterogeneity (reviewed in Kitajima 2007), and ultimately distribution and abundance of adult trees (Condit *et al.* 1995, Wright *et al.* 2003). Seedlings may be fatally damaged by damping-off pathogens (Auspurger 1983, 1984a,b; Augspurger & Wilkinson 2007), consumption and trampling by vertebrates (Osunkoya *et al.* 1992, Weltzin *et al.* 1997, Gillman *et al.* 2002), herbivory (Clark & Clark 1985, Aide & Zimmerman 1990, Benitez-Malvido *et al.* 1999), and litterfall (Scariot 2000, Gillman *et al.* 2004). Although many studies have examined the importance of density- or distant-dependent processes caused by species-specific pests (*e.g.*, Janzen-Connell model; Hyatt *et al.* 2003, Adler & Muller-Landau 2005), less species-specific hazards also restrict where seedlings can establish. Less species-specific hazards, such as litterfall (Facelli & Pickett 1991, Gillman *et al.* 2003a, Peters *et al.* 2004) and vertebrate trampling (Beck 2007), can also generate variation among species in seedling and sapling survival when species differ in their susceptibility to these mortality agents.

Multiple functional traits are thought to affect seedling survival in the shaded forest understory, including seed size (Paz & Martinez-Ramos 2003; Moles & Westoby 2004a,b), cotyledon functional morphology (whether cotyledons are photosynthetic or reserve organs; Ibarra-Manriquez *et al.* 2001, Zanne *et al.* 2005), carbohydrate storage (Canham *et al.* 1999, Myers & Kitajima 2007), and biomechanical properties (Alvarez-Clare & Kitajima 2007). These traits may interact in unison or in trade-off relationships. For example, large reserve-type cotyledons enable investment to physical and

chemical defense and energy storage, which may increase survival. These large seed reserves, however, may also attract vertebrate consumers at the earliest seedling stage, which implies that large-seeded species may initially suffer higher mortality from vertebrate attacks than smaller seeded species.

How do species differences in seedling functional traits explain their differences in susceptibility to various mortality agents during the early seedling stages of tropical trees? This question is best addressed in a natural field setting, yet without confounding density or distance-dependent factors and other environmental factors that may be associated with natural seedshadows near parent trees. From experimental manipulation of seed distribution, Augspurger and Kitajima (1992) found that survival strongly differed in relation to seedling density within a seedshadow, between gaps and shaded understory, and between seedshadow and seedlings transplanted sparsely along a trail. Even though it is rare for seeds to be dispersed far, seedlings established far from other seedlings may be disproportionately important for population dynamics, not only because they are likely to escape density-dependent mortalities but also because they will contribute most to future range expansion (Nathan & Muller-Landau 2000, Jansen *et al.* 2008). Comparison of transplanted seedlings in small common gardens is a viable approach for standardized comparison of species subject to experimental manipulations (*e.g.*, Engelbrecht *et al.* 2005, Myers & Kitajima 2007). By contrast, to investigate some factors that vary stochastically at the scale of the entire forest, quantitative assessment of their importance requires sampling locations over a large area or along multiple long transects extending kilometers.

The first objective of this study was to describe temporal patterns of seedling mortality due to various hazards during the first

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year for eight coexisting tree species in a moist tropical forest. Our second objective was to explore underpinning mechanisms that could lead to the observed patterns. We selected eight common tree species on Barro Colorado Island (BCI), Panama with sufficient seed availability and that encompassed a wide range of seed sizes, shade tolerances, and biomechanical traits (Alvarez-Clare & Kitajima 2007). Specifically, we compared seedling mortality rates and damage susceptibility among the eight species, and related these variables to stem biomechanical strength. Recent studies have shown that species with stronger stems survive better when grown in exclosures in the forest understory (Alvarez-Clare & Kitajima 2007). However, the importance of biomechanical strength for seedling survival has yet to be tested in a field setting where large vertebrates are not excluded. In this study, we identified damage agents and assessed their impact on seedling survival. Furthermore, we examined how stem strength influenced the probability of mechanical damage as well as fatality after receiving a particular type of damage. Seedlings were transplanted to the forest understory at a low density along a 9-km transect at an equivalent ontogenetic stage, and temporal patterns of mortality and damage incidents were analyzed with survivorship curves. To assess the physical disturbance regimes due to litterfall and nonconsumptive animal activities independent of species traits, we placed artificial seedlings constructed of plastic straws and wire along with the real seedlings. Because the artificial seedling approach has been frequently used in other sites to estimate potential damage by litterfall and vertebrate trampling (Clark & Clark 1989, McCarthy & Facelli 1990, Mack 1998, Scariot 2000, Drake & Pratt 2001, Roldan & Simonetti 2001, Gillman *et al.* 2002), we could compare physical disturbance regimes on BCI with other tropical forests. In addition, because we transplanted artificial and real seedlings adjacent to each other, we could assess the efficacy of the artificial seedling method for predicting physical damage and mortality of real seedlings.

We predicted that survival patterns would be characterized by higher mortality during early ontogenetic stages followed by a decrease in mortality after seedlings established in the forest floor. A

potential mechanism for reducing mortality could be an increase in mechanical strength (Alvarez-Clare & Kitajima 2007). Considering the differences in types of hazards affecting seedlings and their differential ability to cope with mechanical damage, we expected that species with high mechanical strength in stems would: (1) be affected less frequently by species-specific damage agents such as herbivory and disease; (2) suffer less intense mechanical damage when affected by any mechanical damage agent; and (3) die less frequently when damaged by herbivores, disease, litterfall, and vertebrate trampling.

METHODS

STUDY SITE AND SPECIES.—This study was conducted in a seasonally moist tropical forest, on Barro Colorado Island (BCI), Panama (9°10' N, 79°51' W). Flora and ecology of BCI are well described in Croat (1978) and Leigh *et al.* (1982). Average annual precipitation is 2600 mm, the majority of which falls between May and December. Also available are community-level comparative studies of tree species regeneration (*e.g.*, Hubbell *et al.* 1999, Wright *et al.* 2005). The experiment took place in young forest (100–300 yr) with abundant palms (mainly *Astrocaryum standleyanum* and *Oenocarpus panamanus*) and evidence of frequent physical disturbances such as tree and branchfalls (K. Kitajima, pers. obs.).

Seeds of eight common tree species were collected on BCI between April and June 2003 and germinated in a shadehouse in 5-cm deep trays filled with forest soil (Table 1). There was substantial variation in germination rate (from *ca* 5 d for *Aspidosperma cruenta* to 36 d for *Eugenia nesiotica*) and size at germination reflecting differences in seed size. The eight study species also varied substantially in biomechanical properties of their stems and leaves including the following: fracture toughness (= work for crack propagation per unit cross-sectional area of break), stiffness measured by modulus of elasticity (MOE), percentage neutral detergent fiber, second moment of area (calculated as $0.25\pi r^4$, where r = radius

TABLE 1. Seed and seedling characteristics of eight tropical tree species used in this study, listed in order of mean seedling survival during 2–6 mo after transplanting in this study and 0–4 mo in vertebrate exclosures located in the forest understory. Mean \pm SD (N) for seed mass excluding seed coat, stem tissue density, and fracture toughness 1 mo after expansion. See Alvarez-Clare & Kitajima (2007) for further details.

Sp. Code	Species	Family	Cot Type ^a	Dispersal	Seed mass (g)	Stem tissue density (g/cm ³)	Stem fracture toughness (J/m ²)
TABR	<i>Tabebuia rosea</i>	Bignoniaceae	PEF	wind	0.035 \pm 0.007 (12)	0.13 \pm 0.03 (25)	1135 \pm 673 (13)
ANAE	<i>Anacardium excelsum</i>	Anacardiaceae	PER	animal	1.811 \pm 0.316 (9)	0.15 \pm 0.03 (45)	1552 \pm 414 (14)
CASE	<i>Castilla elastica</i>	Moraceae	CHR	animal	0.315 \pm 0.005 (8)	0.14 \pm 0.04 (43)	1325 \pm 353 (15)
GUSS	<i>Gustavia superba</i>	Lecythidaceae	CHR	animal	5.566 \pm 1.746 (7)	0.29 \pm 0.04 (46)	5463 \pm 954 (5)
TETP	<i>Tetragastris panamensis</i>	Burseraceae	PER	animal	0.179 \pm 0.026 (10)	0.44 \pm 0.06 (45)	5693 \pm 869 (15)
BEIP	<i>Beilschmiedia pendula</i>	Lauraceae	CHR	animal	2.360 \pm 0.090 (10)	0.35 \pm 0.09 (44)	3430 \pm 637 (15)
ASPC	<i>Aspidosperma cruenta</i>	Apocynaceae	PHR	wind	0.492 \pm 0.002 (6)	0.52 \pm 0.14 (45)	2476 \pm 709 (15)
EUGN	<i>Eugenia nesiotica</i>	Myrtaceae	CHR	animal	0.474 \pm 0.067 (10)	0.47 \pm 0.13 (44)	5433 \pm 1065 (15)

^aCotyledon types follow classification by Garwood (1983); PEF = Phanerocotylar epigeal foliaceous, PER = Phanerocotylar epigeal reserve, PHR = Phanerocotylar hypogeal reserve, CHR = Cryptocotylar hypogeal reserve.

of stem), and flexural stiffness (the product of MOE and second moment of area). See Alvarez-Clare and Kitajima (2007) for a detailed explanation of biomechanical traits.

EXPERIMENTAL DESIGN.—In June 2003, a total of 755 seedlings from the eight study species were transplanted to 100 stations located randomly along a 9 km network of five trails within the young forest on BCI. To determine station locations, we divided the trail into 50-m segments and randomly selected 100 segments. For a given trail, the same side (right or left) was preselected to set stations. The exact location of each station was marked with a plastic flag, which was at least 5 m away from the position along the trail marked by a flagging tape. When the first set of leaves had almost fully expanded, seedlings were removed from the nursery trays and transported in a cooler to the forest. At every station one seedling of each species was planted directly into the ground within 50 cm from the station flag, and marked with a colored plastic toothpick inserted in the ground within 2–3 cm from the stem base. Seedlings were examined every 1–2 wk for 8 mo and every 4 wk for four additional months, for a total of 22 censuses between June 2003 and May 2004. Because of limited seed availability, *Tabebuia rosea* seedlings were transplanted to only 55 of 100 stations. During transplant, microsites were minimally altered (no litterfall or debris were removed). No evident wilting was observed within the few weeks of transplanting, presumably because abundant rainfall during the transplanting period diminished the risk of desiccation. If a seedling died within one week of transplant, however, it was replaced with a new seedling.

In addition to the eight natural seedlings, two artificial seedlings made of plastic and wire were ‘planted’ at each 1-m² station. In total, 200 artificial seedlings were planted, 100 large and 100 small. The design of artificial seedlings followed Clark and Clark (1989), but we used two sizes of artificial seedlings to evaluate the effect of physical disturbance and mechanical damage on seedlings of different size. Each large artificial seedling was made of two 200-mm-long transparent plastic straws oriented in a cross and attached together with staples. A stiff, 3-mm-diameter × 100-mm-long wire was inserted 20 mm into the vertical straw and the remaining 80 mm into the ground to simulate a root (Fig. 1 in Clark & Clark 1989). Small artificial seedlings were constructed in the same way, except that they were made from 100-mm-long straws and 50-mm-long wire ‘root.’

SURVIVAL AND DAMAGE TYPES AND AGENTS.—At every census, each seedling was examined for particular types of damage and classified as dead or alive. A seedling was considered dead when it was completely dried, when the stem was cut in two and the lower portion uprooted, or when the whole seedling was missing. We classified damage types in two ways, by apparent damage agents (vertebrate activity, litterfall, and disease) and by types of mechanical damage (leaf damage, stem bent, stem broken, and seedling uprooted).

The three damage agents were not mutually exclusive; seedlings often died after being affected by two or more agents. Our intention was not to determine the ultimate cause of mortality, but to describe

the temporal patterns of the damage agents that may synergistically kill seedlings. ‘Vertebrate activity’ was characterized by plants that were completely missing or by plants that were uprooted, flattened, or had damaged, broken, or chewed stems with no evidence of falling leaves or branches. Chewed stems, missing cotyledons, and uprooted seedlings suggested that vertebrate activity was mostly consumption-related rather than trampling. Damage by ‘litterfall’ was recorded when a seedling had a bent, damaged, or broken stem and there was direct evidence of litterfall or debris above it. Seedlings were categorized as ‘diseased’ when they exhibited necrotic tissue or when they were severely wilted with at least one dry leaf albeit moist soil. An artificial seedling was considered damaged when it was bent such that at least one of its arms was touching the ground, when it was flattened, cut, chewed, or missing (Clark & Clark 1989). For comparison with previous studies, three standardized categories of damage were recorded for artificial seedlings: vertebrate activity, litterfall, and unknown. An artificial seedling was classified as damaged by vertebrate activity if it was flattened, chewed, cut, or missing with no evidence of litter or branchfall that could have caused the damage. If an artificial seedling was bent or flattened, with evidence of litterfall or debris above it, it was considered damaged by litterfall. If a seedling was bent but there was no obvious cause, it was classified as unknown.

For real seedlings, we also recorded the four types of mechanical damage that would potentially lead to fatality: leaf damage, stem bent, stem broken, and seedling uprooted. A leaf was considered damaged if it was fractured, incomplete, or had missing sections > 10 percent of the leaf area. A seedling with at least one damaged leaf was classified with ‘leaf damage.’ A stem was considered ‘bent’ if it was curved or tilted at least 45°, and ‘broken’ if it was fractured in two or more sections. A seedling was uprooted when it was completely pulled from the ground. Although the four damage types were not mutually exclusive, only the first type that occurred to a given plant was used for calculation of ‘damage fatality’ at the end of the study. For each type of mechanical damage (M_x), we calculated the likelihood of dying after receiving a given type of damage $P(D | M_x)$ expressed as the following formula (Gotelli & Ellison 2004):

$$P(D | M_x) = \frac{P(D \cap M_x)}{P(M_x)}, \quad (1)$$

where $P(M_x)$ is the probability of receiving the damage type M_x , and $P(D)$ is the probability of death. The conditional probability $P(D | M_x)$, multiplied by 100 and expressed in percent, was called ‘percent damage fatality.’ We also evaluated the relationship of seedling survival (% survival from 0 to 2 mo and 2–6 mo) and percent damage fatality with material and structural stem properties (e.g., MOE and fracture toughness), which were measured in a different study (Alvarez-Clare & Kitajima 2007).

STATISTICAL ANALYSES.—Temporal patterns of the occurrence of seedling death and damage agents were analyzed using Kaplan–Meier survival distribution functions (Collett 2003). Survival functions describe the probability that an individual survives longer than a specified period, considering individuals at risk at the beginning

of each interval and excluding censored values. An interval is defined as the lapse between two mortality events. To examine changes in seedling survival probability with time and among species, we constructed Kaplan–Meier survival functions for 0–2, 2–6, and 6–12 mo periods as well as for the entire 0–12 mo period. The log-rank test and the Wilcoxon test (Pyke & Thompson 1986, Collett 2003) were used to compare these survival functions, but only results from log-rank tests are reported because the two tests yielded very similar results. We tested for differences in seedling survival probabilities among species at the end of a specified interval and for differences in overall seedling survival probabilities between time intervals using Spearman rank correlation coefficients. For interspecific comparison within types of damage, likelihood chi-squared tests were used. Lastly, Spearman correlations were calculated to examine relationships of species biomechanical and ecological traits with percent damage fatality and percent survival (*i.e.*, no. of seedlings alive at the end of an interval/ no. of seedlings alive at the beginning of the interval \times 100). Analyses were performed using JMP IN 4.0 (SAS Institute Inc., Cary, North Carolina). In all figures, one and two asterisks indicate significance at the $\alpha = 0.05$ and $\alpha = 0.001$ level, respectively. The first three sections of the results and discussion refer to real tree seedlings and the fourth section refers to artificial seedlings.

RESULTS

TREE SEEDLING SURVIVAL.—During the first 2 mo, 59 percent of the transplanted seedlings died, and by the end of 1 yr, 76 percent of transplanted seedlings were dead. The temporal pattern of mortality and overall percent mortality at the end of the first year differed among species. Survival distribution functions varied among species for the entire 1-yr period and for each shorter interval (Fig. 1). Species rankings of survival probability changed among intervals. Seedling survival during the 0–2 mo period was uncorrelated with survival either during the 2–6 mo or the 6–12 mo period (Table 2). Because of high seedling mortality during 0–2 mo, overall survival for the 0–1 yr interval still largely reflected 0–2 mo survival (Fig. 1A,D), rather than survival after 2 mo (Fig. 1B,C).

DAMAGE AGENTS AFFECTING TREE SEEDLINGS.—Survival functions (time-course of the probability to remain unaffected) differed significantly among damage agents for all species combined, with vertebrate activity being the most common damage agent especially during 0–2 mo (Fig. 2A). In addition, damage agents affected species differentially. The percentage of seedlings affected by vertebrate activity after 1 yr ranged from 42 percent for *A. cruenta* to 87 percent for *Beilschmiedia pendula* (Fig. 2B). Likewise, species were affected

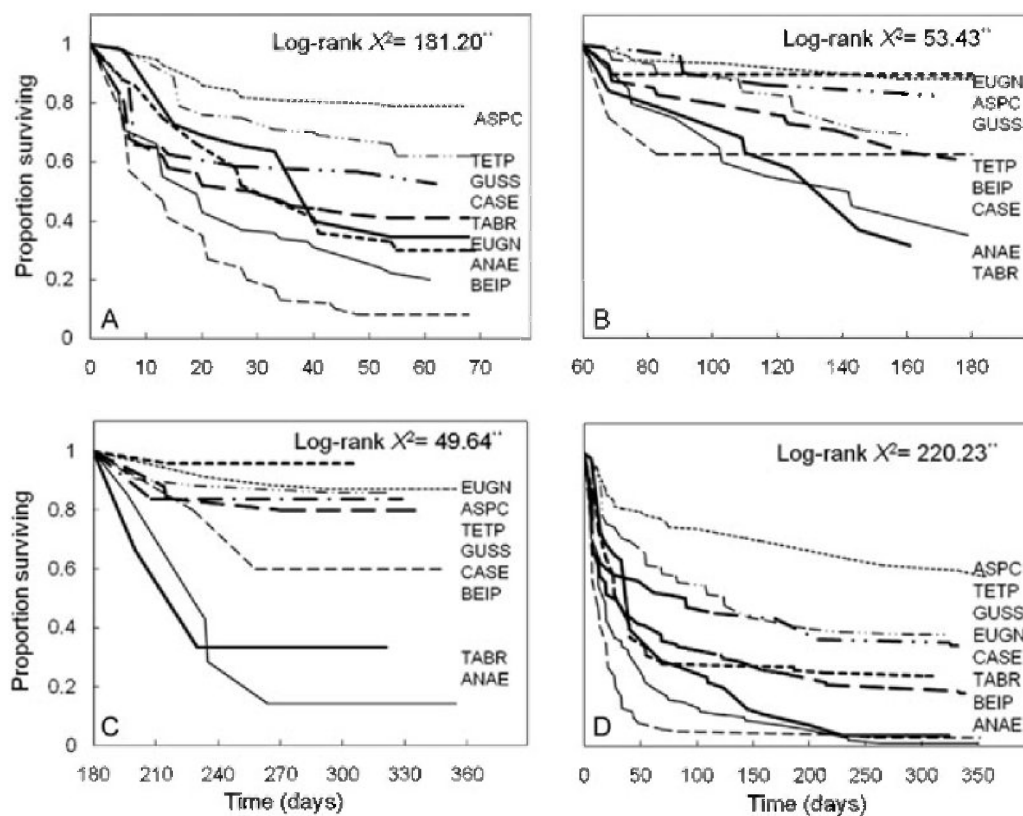


FIGURE 1. Kaplan–Meier survival functions for seedlings of eight tree species (indicated by the species code at the tail end of each curve). Proportion surviving is relative to the number of seedlings alive at the beginning of each interval: (A) 0–2 mo; (B) 2–6 mo; (C) 6–12 mo; and (D) total study period (0–12 mo). Refer to Table 1 for the full species names. ** $P < 0.001$ for difference among curves by log-rank tests.

TABLE 2. Concordance of ranks of seedling survival probabilities for eight tree species between the specified intervals (Fig. 1). Shown are Spearman correlation coefficients with P-values in parentheses and significant ($P < 0.05$) correlations in bold.

	0–2 mo	2–6 mo	6–12 mo
2–6 mo	0.50 (0.207)		
6–12 mo	0.55 (0.160)	0.90 (0.002)	
0–12 mo	0.90 (0.002)	0.79 (0.021)	0.83 (0.010)

differently by disease; in the species most susceptible, *Anacardium excelsum*, > 80 percent of seedlings showed disease symptoms after 8 mo (Fig. 2C). Only 4.1 percent of the seedlings were affected by litterfall, with no interspecific differences in damage agent distribution over time (log-rank $\chi^2 = 13.7$, $df = 7$, $P > 0.05$; data not shown).

TYPES OF MECHANICAL DAMAGE RECORDED ON TREE SEEDLINGS.—

Four types of mechanical damage were recorded during 1 yr: damaged leaves, bent stems, broken stems, and uprooted seedlings. At the end of the study, 77 percent of seedlings showed some form of damage; 30.6 percent of the seedlings exhibited leaf damage (especially missing leaf pieces or edges characteristic of invertebrate herbivory), 28.7 percent had broken stems, 23.9 percent had bent stems, and 25.6 percent had been uprooted (Table 3). These categories were not mutually exclusive. For example, 45 percent of the damaged seedlings had two or more types of damage.

Species did not differ in the likelihood of suffering mechanical damage. However, species differed in their likelihood to die after suffering leaf damage, bent stems, or being uprooted but not having their stem broken. Percent damage fatality (as defined by Equation 1) differed among species following leaf damage ($\chi^2 = 78.6$, $df = 7$, $P < 0.001$; Table 3), and stem bending ($\chi^2 = 57.3$, $df = 7$, $P < 0.001$; Table 3). In contrast, there was no significant interspecific difference in damage fatality subsequent to uprooting ($\chi^2 = 14.0$, $P = 0.052$; Table 3) and broken stems ($\chi^2 = 9.4$, $P = 0.226$; Table 3), probably because this type of damage was usually fatal in all species. However, due to relatively low number of uprooted and broken seedlings in some species (cell $N < 5$), results should be interpreted with caution. Across the eight species, percent seedling survival or percent damage fatality was correlated with two of the biomechanical properties of seedlings (Table 4); stem toughness and stem tissue density were positively correlated with percent survival 2–6 mo after transplant. Similarly, percent fatality following stem breakage or bending was negatively correlated with stem toughness and stem tissue density. Second moment of area, a function of stem diameter, was positively correlated with damage fatality following stem breakage (*i.e.*, large seedlings were more likely to die following stem breakage), but had no relationship with overall seedling survival.

ARTIFICIAL SEEDLINGS.—Large and small artificial seedlings did not differ in their damage frequencies ($\chi^2 = 6.0$, $df = 3$, $P = 0.111$), and

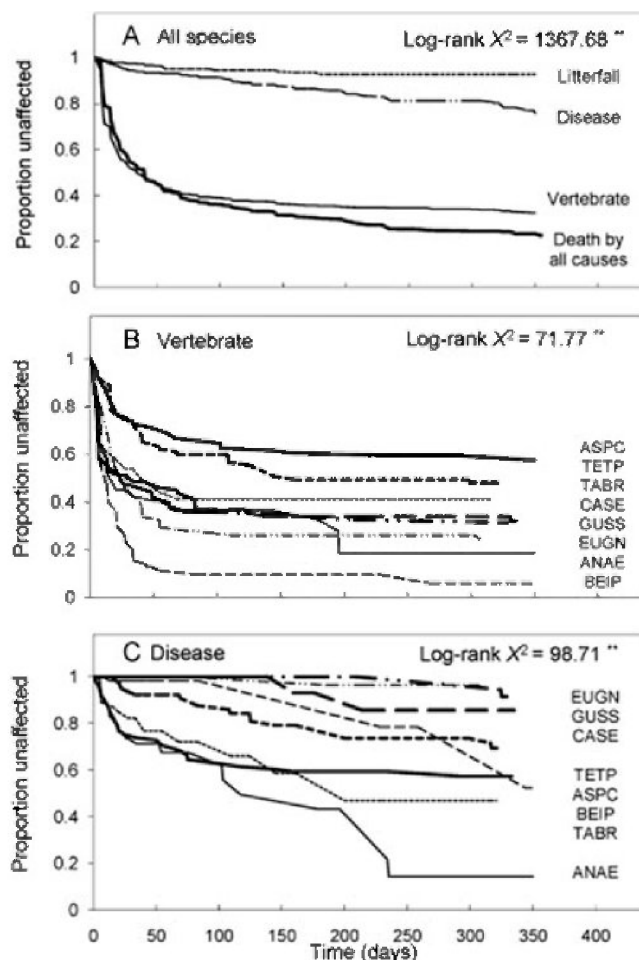


FIGURE 2. Kaplan–Meier survival functions (proportion of seedlings yet to be affected by specified damage agents plotted against time) for eight tree species transplanted to the forest understory. (A) Comparison of damage agents for the pooled data of the eight study species. These damage agents are not mutually exclusive; a given seedling may be affected by multiple damage agents. Also shown is survival function for all species pooled together. Shown separately for individual species are: (B) proportion of seedlings yet to be affected by vertebrate activity, indicated by symptoms including uprooted, flattened, chewed or broken stems, or missing seedlings; and (C) proportion yet to show symptoms of disease. ** $P < 0.001$ for difference among curves by log-rank tests.

therefore they were averaged for comparisons with real seedlings and with previous studies. After 1 yr, 9.5 percent of artificial seedlings were damaged by litterfall, 15.5 percent were damaged by vertebrate activity, and 22.5 percent were bent by unknown causes. Damage levels on artificial seedlings were within the range of damage reported for other sites (Table S1). Overall, real seedlings were damaged more than artificial seedlings (Fig. 3). Artificial seedlings were damaged more by litterfall and by unknown causes, and less by vertebrate activity than real seedlings of any species.

Temporal patterns for each type of damage differed between artificial and real seedlings (Fig. 4). Vertebrate activity damaged a much higher proportion of real than artificial seedlings, especially

TABLE 3. Percent damage fatality (% of seedlings that died after receiving a given type of mechanical damage) for eight tree species during 1 yr after transplant to the forest understory. N = total number of seedlings affected by each damage type. Damage types are not mutually exclusive. For species codes refer to Table 1.

Sp. code	Stem broken		Uprooted		Stem bent		Leaf damage	
	N	Percent fatality	N	Percent fatality	N	Percent fatality	N	Percent fatality
TABR	4	100.0	22	95.5	20	100.0	20	100.0
ANAE	36	100.0	36	100.0	39	100.0	37	97.3
CASE	31	100.0	16	100.0	17	76.5	45	64.4
BEIP	22	100.0	16	100.0	9	88.9	7	100.0
GUSS	36	94.4	35	85.7	25	48.0	47	44.7
TETP	19	94.7	9	100.0	19	57.9	35	48.6
EUGN	44	93.2	41	97.6	28	57.1	9	22.2
ASPC	25	92.0	18	88.9	23	47.8	31	32.3
All species	217	96.3	193	95.3	180	72.2	231	61.5

TABLE 4. Relationships of stem biomechanical traits with % survival in the field during two periods and with % damage fatality following stem breakage and bending. Percent damage fatality = percent of seedlings that died after receiving a given type of mechanical damage. Spearman correlation coefficients for species means (N = 8) with P values in parentheses and significant correlations in bold.

Biomechanical traits ^a	Percent Survival		Percent damage fatality	
	0–2 mo	2–6 mo	Stem broken	Stem bent
Toughness (J/m ²)	0.46 (0.294)	0.96 (0.001)	-0.77 (0.044)	-0.82 (0.024)
Density (g/cm ³)	0.57 (0.139)	0.86 (0.007)	-0.79 (0.019)	-0.71 (0.048)
Percent neutral detergent fiber	0.26 (0.531)	0.69 (0.058)	-0.406 (0.318)	-0.69 (0.056)
Modulus of elasticity (MN/m ²)	0.36 (0.385)	0.64 (0.086)	-0.36 (0.388)	-0.61 (0.108)
Second moment of area of stem (mm ⁴)	-0.48 (0.233)	-0.62 (0.102)	0.79 (0.019)	0.61 (0.108)
Flexural stiffness (N cm ²)	-0.19 (0.651)	-0.14 (0.736)	0.61 (0.109)	0.54 (0.169)

^aBiomechanical traits were determined 1 mo after leaf expansion, as reported in Alvarez-Clare & Kitajima (2007) and Table 1. See methods for brief descriptions of these traits.

during the first 50 d (Fig. 4A). Real seedlings were particularly vulnerable to vertebrate activity during the first 2 mo after transplant, but artificial seedlings received a more constant rate of vertebrate damage throughout the year. Litterfall damage was similar for both artificial and real seedlings. In both cases < 10 percent

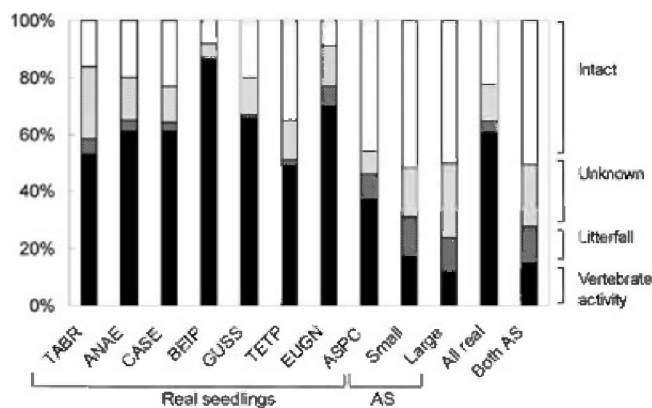


FIGURE 3. Percent of real and artificial seedlings (AS) damaged during 1 yr in the forest understory by specific damage agents. Categories are mutually exclusive, as each seedling was assigned only to the first damage agent it experienced. Small and large artificial seedlings were pooled as 'both AS' (N = 200), and real seedlings were pooled as 'all real' (N = 755). For species codes refer to Table 1.

of the seedlings were affected (Fig. 4B). At a given station, whether at least one real seedling was damaged by litterfall was independent of the number of artificial seedlings hit by litterfall as well as whether at least one artificial seedling was damaged ($P > 0.05$, logistic regression in all species after Bon-Ferroni correction). In addition, survival functions describing the proportion of seedlings damaged by unknown causes did not differ between artificial and real seedlings (Fig. 4C).

DISCUSSION

TEMPORAL PATTERNS OF SURVIVAL AND DAMAGE AGENTS.—Various damage agents and differential susceptibility among species to damage created a complex temporal pattern of seedling survival in the forest understory, resulting in reversals of survival ranks among species between early and later periods. Consistent with other studies (Augspurger 1984a, Kitajima & Augspurger 1989, De Steven 1994), seedling mortality was highest during the first 2 mo after transplant, decreasing gradually and then becoming more constant over time in all species (Fig. 1). However, mortality rates changed differently for different species after 2 mo, such that survival ranks during the 2–6 and 6–12 mo intervals were completely independent of those during the 0–12 mo interval. Some of the rank reversals could be explained by improved survival of some of the large-seeded species (e.g., *E. nesiotica*, *B. pendula*) after initial high mortality due to vertebrate cotyledon consumption. However, another large-seeded species, *A. excelsum*, exhibited poor survival throughout the year. This difference could be due to species differences in stem development during ontogeny. For example, *Eugenia* and *Beilschmiedia* significantly increased stem toughness between 1 and 6 mo, whereas *Anacardium* did not (Alvarez-Clare & Kitajima 2007).

Vertebrates were the most common type of damage agent, mainly during the 0–2 mo period and particularly for large-seeded

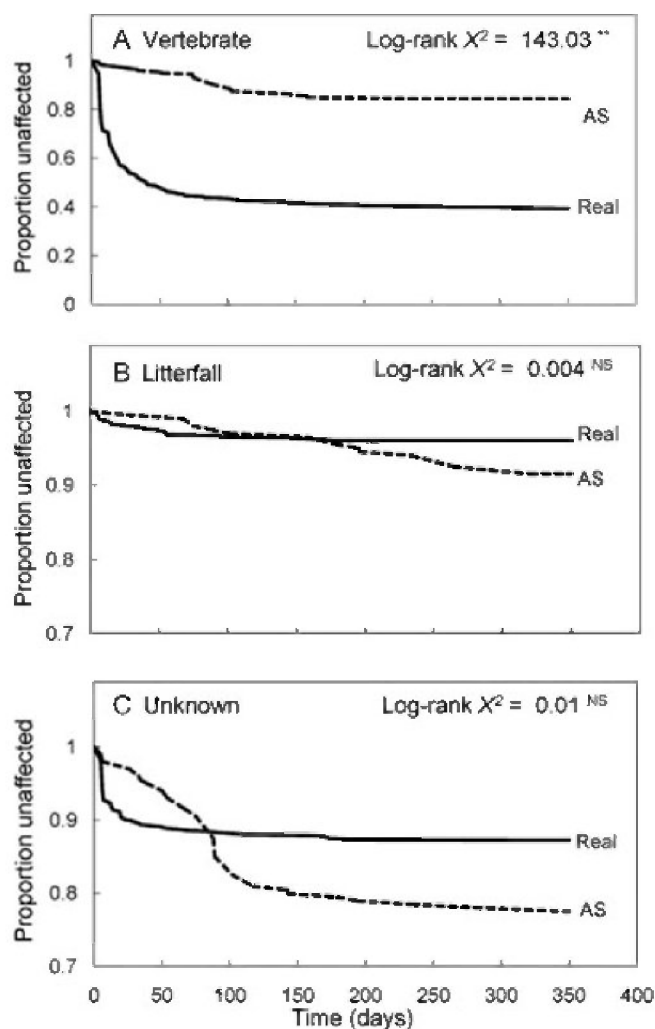


FIGURE 4. Kaplan–Meier survival functions (proportion of seedlings yet to be hit by a particular hazard type) for artificial seedlings (AS) and real seedlings during 1 yr in the forest understory for three types of hazards: (A) vertebrate activity; (B) litterfall damage; and (C) bent or broken by unknown causes. For real seedlings, the average from eight species is shown. Note the different scales in y-axes. ** $P < 0.001$, NS $P > 0.05$, for difference among curves by log-rank tests.

species with large reserve-type cotyledons (Table 1; Figs. 2A,B). Although vertebrate activities included nonconsumptive actions such as trampling, cotyledon predation was the primary cause of mortality, as indicated by high percentages of seedlings uprooted but left partially uneaten with stems cut in half and cotyledons missing. Interestingly, leaf damage by insect herbivory was very low during this period. Predation of large storage cotyledons by seed-eating mammals is very common on BCI (Sork 1987, Molofsky & Fisher 1993, Asquith *et al.* 1997). In fact, due to absence of top predators and protection from hunting seed-eating mammals, such as agoutis (*Dasyprocta punctata*), collared peccaries (*Tayassu tajacu*), squirrels (*Sciurus granatensis*), and spiny rats (*Proechimys semispinosus*),

exist at much higher densities on BCI than in other Neotropical forests (Terborgh & Wright 1994; Wright *et al.* 2000, 2007). When *Tachigali versicolor* seeds were planted every 5 m along a 6-km transect, seed and seedling mortality due to vertebrate consumption was also high (Augspurger & Kitajima 1992). Not only seed predator abundance but also seed and seedling density and seedling age may influence predation by vertebrates. Within a dense natural seedshadow, territorial seed predators, such as agoutis, may be satiated to cause lower mortality of seeds and seedlings (Kitajima & Augspurger 1989). Gillman and Ogden (2003b) recorded much lower vertebrate activity on naturally established seedlings older than those used in our study.

Throughout the study period, damage by vertebrate predation showed a different pattern than damage by disease. Whereas vertebrate damage affected more than 60 percent of seedlings during the first 2 mo but very few after this period, disease affected seedlings at a constant rate (Fig. 2A,B). As seed reserves become exhausted and stems become tough during seedling development, seedlings become less attractive as food. For *T. versicolor* on BCI, a wind-dispersed species with relatively large seeds (*ca.* 0.5 g), vertebrate predation was the major mortality agent during the first two weeks after germination, but disease incidents increased afterwards (Kitajima & Augspurger 1989). Augspurger (1984a), who studied naturally germinated seedlings of nine wind-dispersed species (which tend to have smaller seed size than animal dispersed species) found overall high mortality from disease. The two species overlapping between the current study and her study exhibited similar levels of mortality at the end of 2 mo (20 vs. 27% for *A. cruenta* and 65% for *T. rosea* in both studies).

Many comparative studies found that large seed size improves survival in shade (Osunkoya *et al.* 1992; Moles & Westoby 2004a,b) because large seed reserves enable resprouting (Green & Juniper 2004) and energy availability for better defense response and repair of damaged tissues (Kitajima 2007, Myers & Kitajima 2007). Ample reserves should also facilitate construction of strong tissues. However, combined results from experiments realized in ‘field settings’ (this study) and in common gardens where vertebrates are excluded (Alvarez-Clare & Kitajima 2007) reveal that large seed reserves used to build strong stems (*i.e.*, physical defense) become important only under conditions in which vertebrate seed eaters are excluded, or after seedlings escape seed-eating mammals.

FATALITY FOLLOWING DIFFERENT TYPES OF DAMAGE.—Differences in survivorship among species reflected the interaction between the likelihood of damage and the ability to tolerate damage-induced stress. Contrary to our expectation, species with greater material strength (species with greater stem toughness and stiffness) did not experience lower probability of mechanical damage, but once damaged they were less likely to die (Table 4). It is possible that species with stronger stems were damaged less severely, thus more likely to recuperate after damage. However, detailed observations evaluating the intensity of damage in stem tissues would be required to confirm this explanation. An exception was *A. cruenta*, a shade-tolerant species with low stem toughness, which was the least likely to die after suffering mechanical damage. Perhaps other factors,

such as carbohydrate storage reserves in stems and roots (Myers & Kitajima 2007) and secondary defense chemicals (Harper *et al.* 1993) play an important role in the ability of seedlings to tolerate mechanical damage. The probability of dying after being damaged (measured as percent damage fatality) was different depending on the type of damage received (Table 3). Our results as well as those of Marquis and Braker (1994) suggest that leaf damage (caused by insect herbivory) is less fatal than stem bending, breakage, and uprooting.

ARTIFICIAL SEEDLINGS.—Because artificial seedlings are not significantly consumed by vertebrates, they are accurate predictors of mechanical damage only in environments where indiscriminate, nontrophic damage agents (*e.g.*, litterfall, vertebrate trampling) are more frequent than trophic interactions. Gillman *et al.* (2002) monitored 1200 artificial seedlings placed adjacent to real seedlings and concluded that only litterfall damage, but not damage due to vertebrate activities, could be predicted by artificial seedlings. We also found different temporal patterns of damage due to vertebrate activities between real and artificial seedlings, whereas temporal patterns of litterfall damage were similar between real and artificial seedlings (Fig. 4). The lack of significant station-by-station association of litterfall damage between artificial seedlings and real seedlings in our study may be due to low sample size (100 stations).

Are artificial seedlings useful for comparisons of microdisturbance regimes among different forests? The total percentage of artificial seedlings damaged on BCI after 1 yr was within the range reported in other studies (Table S1). However, the percentage ($9.5 \pm 2.1\%/yr$) of artificial seedlings damaged by litterfall on BCI was lower than the percentage reported in most other tropical forests, possibly due to differences in rainfall, canopy composition, and topography (van der Meer & Bongers 1996, Gillman *et al.* 2004). La Selva, Costa Rica (Clark & Clark 1989) exhibited the highest percentage of damaged artificial seedlings (82.4%/yr), and the intensively hunted forest in Beni, Bolivia (Roldán & Simonetti 2000) the lowest (25%/yr). However, in the Bolivian study, litterfall damage was not recorded. This large variation among forest communities reflects the abundance of factors influencing seedling survival, such as seasonal patterns of litterfall and abundance of large vertebrates that cause trampling damages.

CONCLUSIONS.—This study evaluated the relative importance of various types of damage that affect survival of seedlings using an experimental design simulating the fate of seedlings dispersed farther from the dense seedshadow near the parents, comparing species at standardized ontogenetic stages. Survivorship analyses revealed that diverse ecological pressures, such as vertebrate predation and disease affect seedlings differentially through time and among species. Additionally, differences among damage agents affecting real and artificial seedlings indicate that mechanical damage caused by litterfall may become significant only after the early establishment period. Although there was no relationship between biomechanical strength and the types of mechanical damage affecting each species, species with stronger stems survived better after the initial 2 mo and after receiving mechanical damage, suggesting that investment

in strong stems is beneficial for seedling performance. Thus, the seedling's ability to persist in the shaded understory is enhanced by biomechanical strength, not because it results in lower chance of being damaged, but more likely because of less severe damage for species with strong materials.

The high mortality of seedlings due to vertebrate consumption of cotyledons during initial seedling establishment on BCI provides insight into the ecological input of faunal change due to hunting and fragmentation. Lack of hunting and rarity of carnivorous animals on BCI explain the high abundance of seed-eating mammals (Wright *et al.* 2000) and the high mortality of seedlings with large cotyledons found in this study. In contrast, abundance of seed-eating mammals has declined drastically due to hunting and habitat fragmentation in many tropical forests (Redford 1992). This mammal decline could lead to dominance of large-seeded species and thus long-term changes in species composition in human-influenced tropical forests (Muller-Landau 2007).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

TABLE S1. *Percentage of artificial seedlings affected by specified damage agents in this and other published studies in different forest communities.*

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